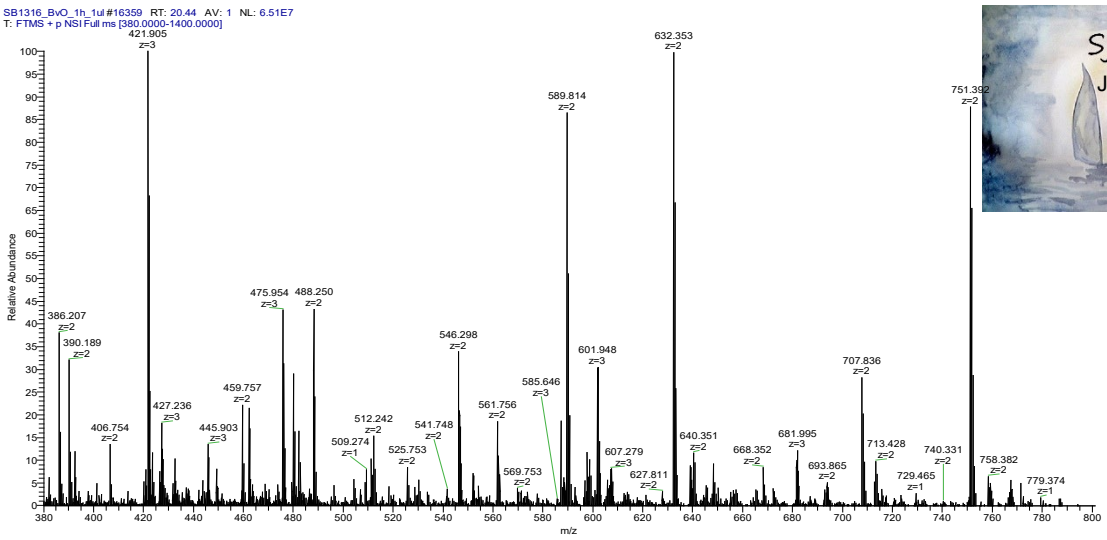
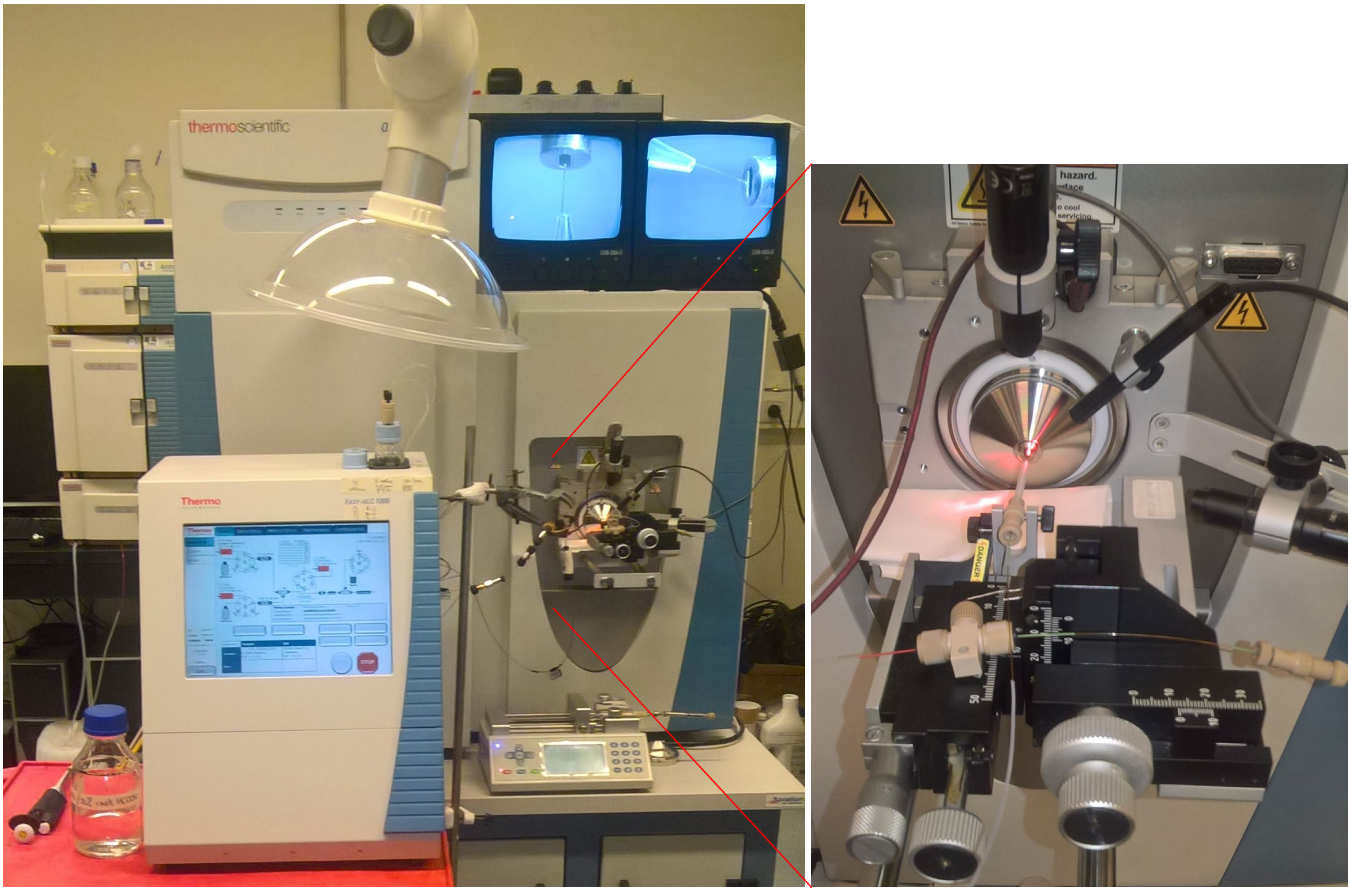
Proteomics at WUR Biochemistry

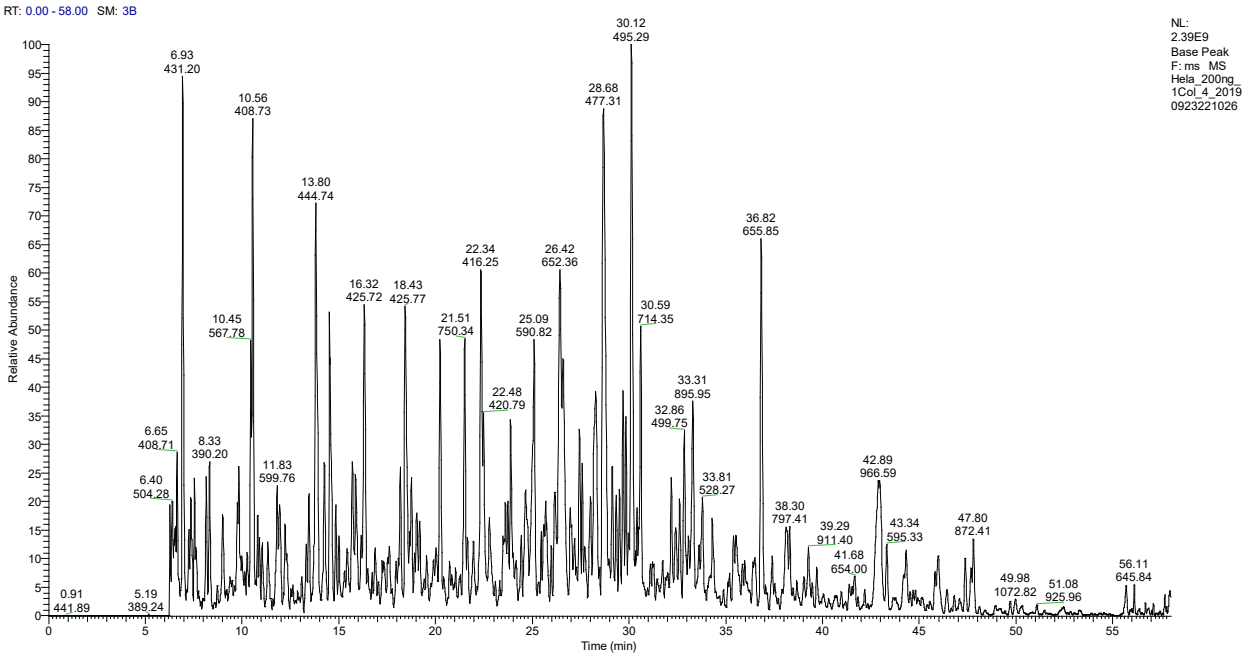


A) General information

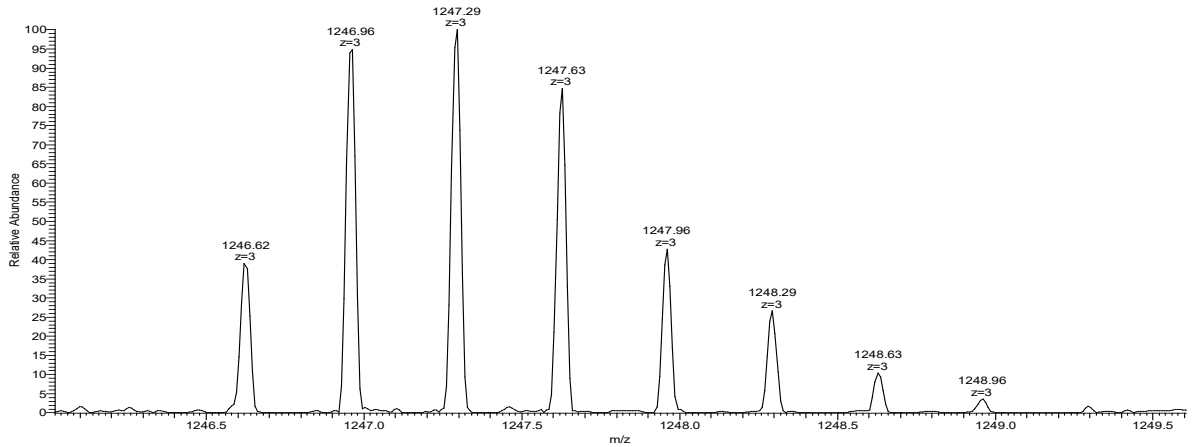
High quality protein identification as well as accurate relative protein quantitation is done by nanoLC-MSMS. Reversed phase nano LC (Thermo nLC1000) using home made capillary columns (1.9 µm particles) results in peptide separations with a high resolution. MS spectra of the peptides are measured with a Q Exactive-HFX at approximately 5 ppm deviation or less. After each MS scan, MSMS spectra of the peptides are acquired when enough peptide is available (ca 20 scans/s). All measurements combined yield optimal protein identifications and relative quantitation.



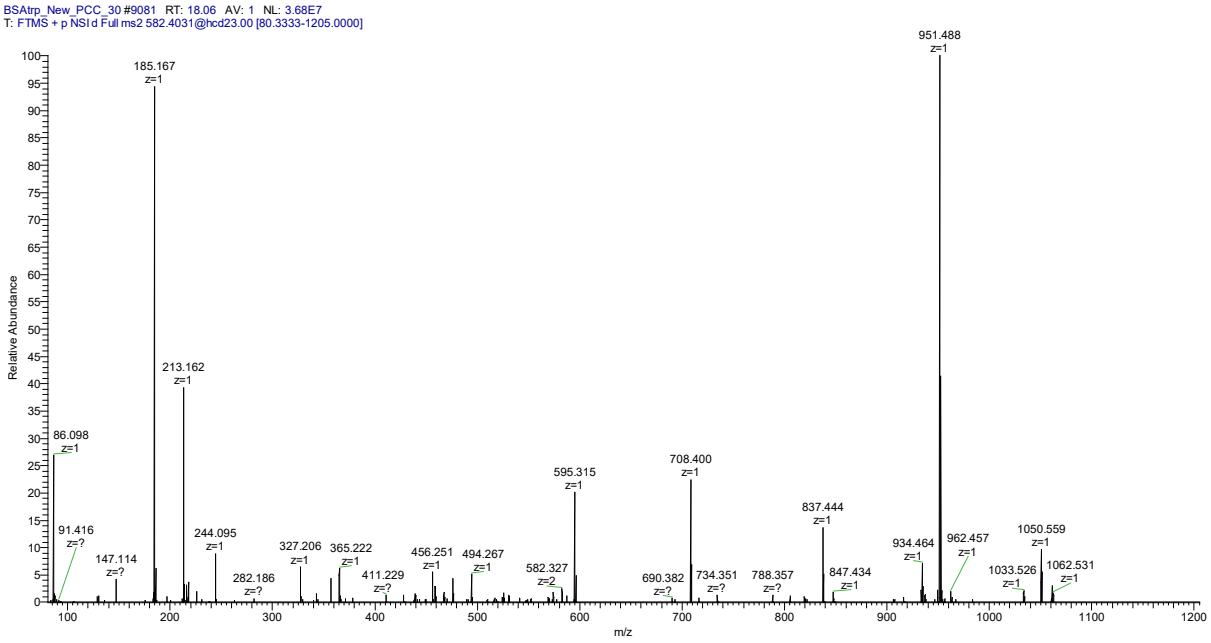
(Hela cell line) Peptide separations at a high chromatographic resolution:

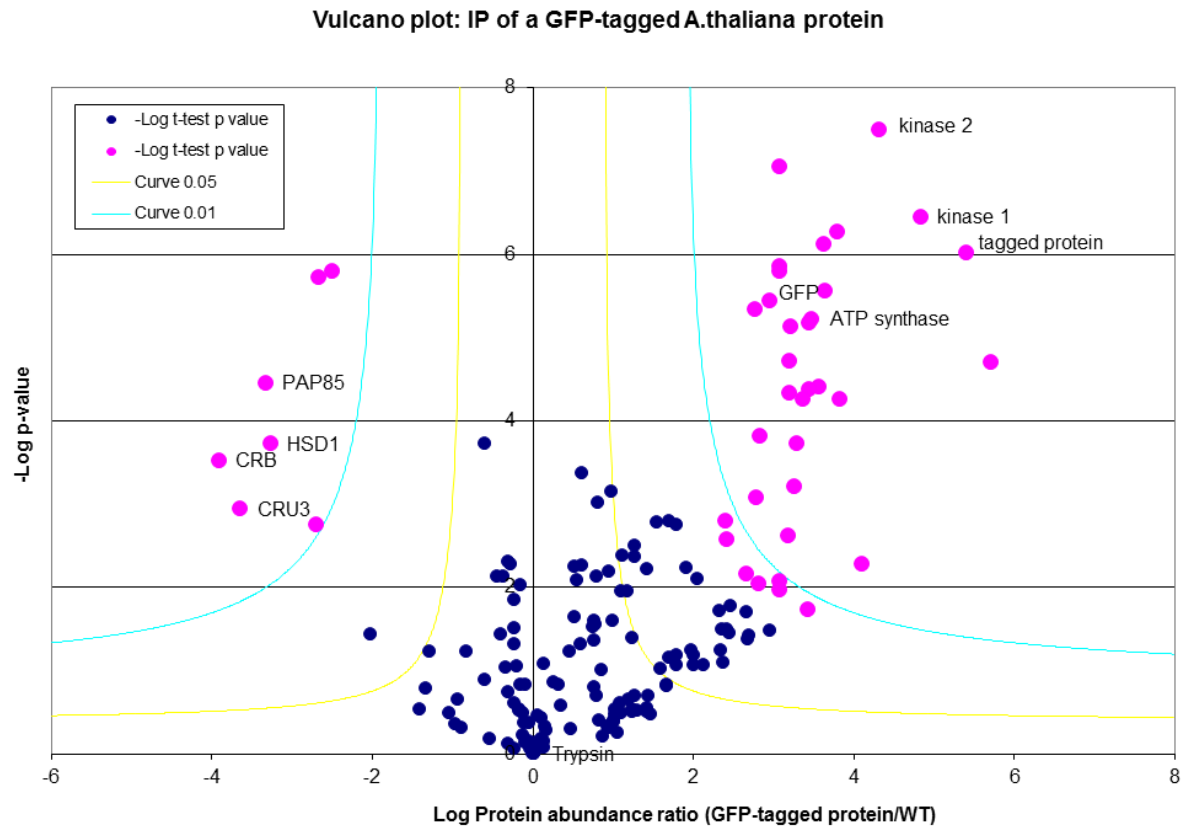


High resolution Orbitrap MS spectra

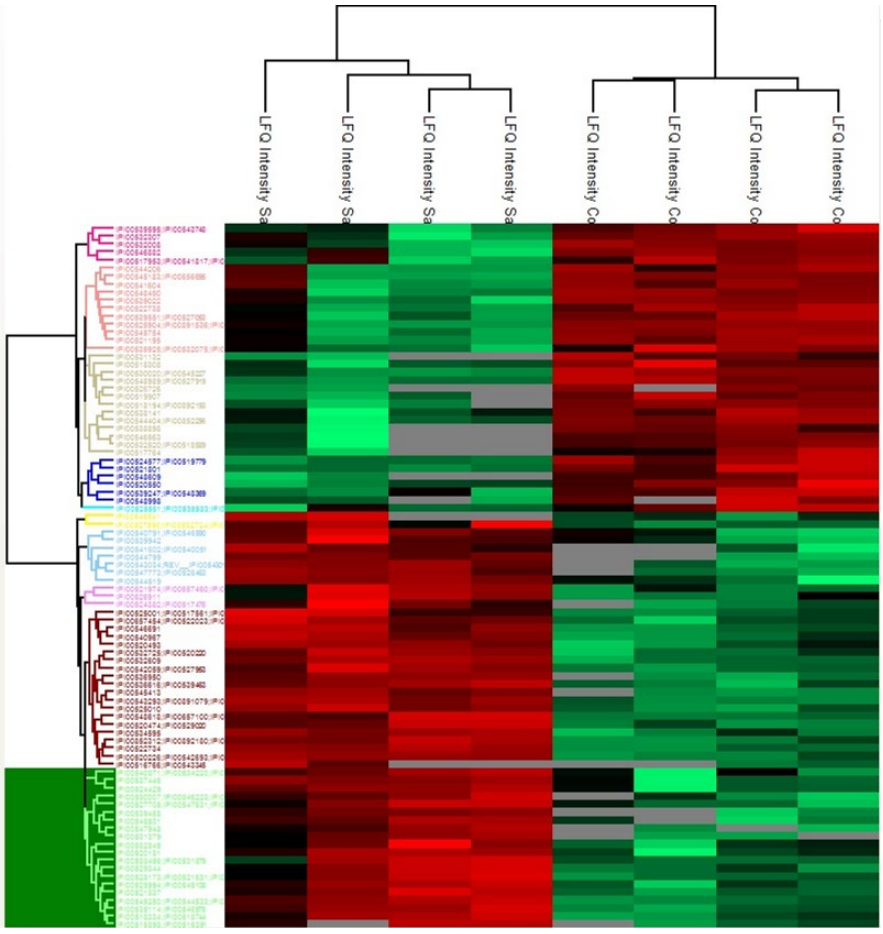


And high resolution MSMS peptide fragmentation spectra





Proteomics data clustering

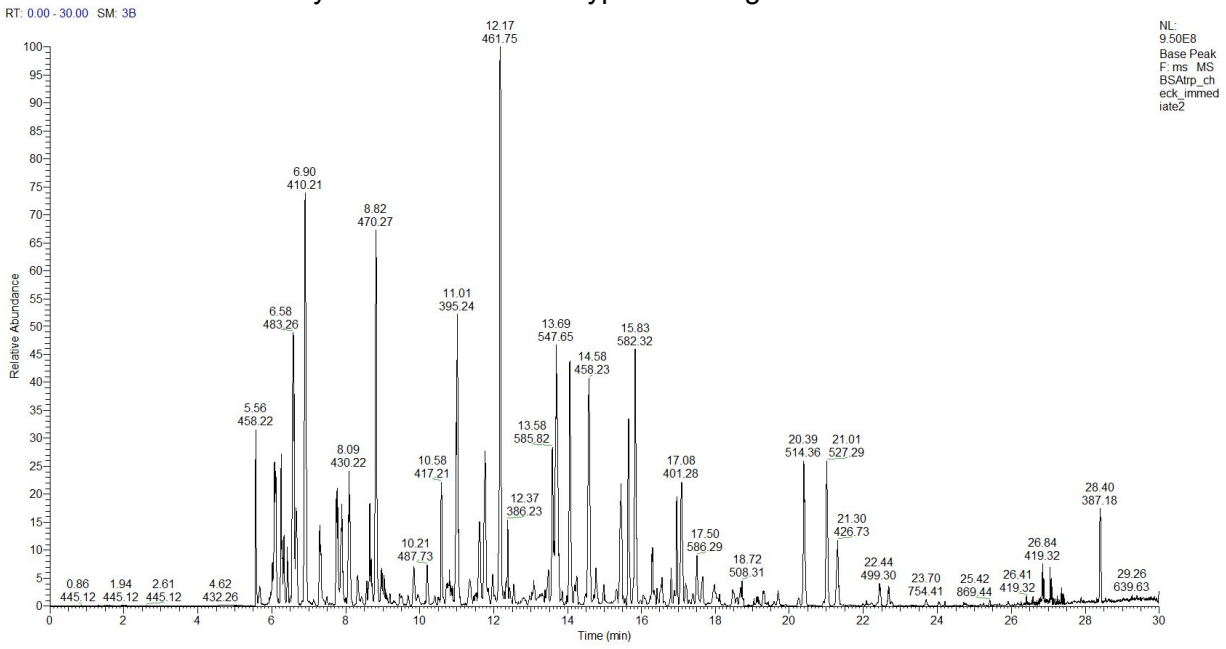


B) Practical proteomics information

Maximally 5 ul of sample will be injected per LC-MS run. Minimally 25 ul peptide solution has to be handed in optimally containing 0.1-0.2 ug/ul peptide. All peptide samples need to be prepared via the FASP procedure or have to be cleaned via the µColumn cleanup procedure at pH 3. Please see our Proteomics Sample Preparation Protocol (https://www.wur.nl/en/Expertise-Services/Chair-groups/Agrotechnology-and-Food-Sciences/Laboratory-of-Biochemistry/Facilities.htm) for details how to prepare proteomic samples.

Before every measuring series, the system [nLC1000 (Thermo EASY nLC) + MS + MSMS] is checked by measuring a standard BSA digest. Points that are checked include LC peak width as well as MS and MSMS sensitivity and spectral quality. Also, no peptides from previous injections should be visual. When everything is alright, and only then, the next sample set will be injected with a “fast” cleaning gradient directly after each measurement gradient.

System check with a tryptic BSA digest



Gradient	Sample type	Number of samples that can be measured per day
Fast gradient	Simplified mixtures (e.g. Immuno Precipitations or In Gel Digests)	16
Standard gradient	Complex mixtures (e.g. FASP prepared complete proteomes)	10

When the standard gradient is used, the total measuring time (including cleaning gradient) is 2 hours per sample. Because of the checks before and some cleaning gradients after each sample set, 10 samples can be measured per day using the standard gradient. It is advised to put the Controls/Blancs first in your sample list, followed by the Samples. When less than the maximal amount of samples is handed in, then the remaining time will be filled with cleaning gradients. Minimally one complete measurement day will therefore always be used and charged. When the system is so heavily contaminated that it cannot be cleaned within the same day (e.g. due to high concentrations of detergents) then an extra measurement day can be charged.

For identification and relative quantitation, the MaxQuant software package will be used. When a database is not publicly available, than a database in fasta format has to be handed in before measurements will be done. Search times necessary to compare the data to the database strongly depend both on the number of LCMS runs as well as on the database size. When a species specific database is used for trypsin digests, it will take about 0.5 to 1 hour per run. The MaxQuant search result (a table with identification + normalized intensities) will be filtered with a filtering and statistics software called Perseus leaving confident identifications only. When applicable, a Vulcano plot will be added (see page 3), as well as significance info and e.g. hierarchical clustering when asked for. Significance info is only available for experiments done at least in triplicate. To be able to do it well, it is advised to do the experiments in fourfold with real biological replicates (not just technical replicates). The Perseus filtered data as well as the original MaxQuant data [+ the protein abundance ratio Graph] will be supplied to you.

An example Vulcano plot of a graph is shown on the top of page 3. In the plot, the p-values are shown on the Y-axis as –Log p (higher is more reliable). The X-axis shows the ratio of the average protein Label Free Quantitation intensities between each data set, e.g Sample versus Control (on a logarithmic scale as well). Proteins whose average concentration significantly differs between the two data sets are shown with pink dots. Proteins that do not vary between the two conditions are shown with blue dots.

One remark. Since MaxQuant uses peak intensities for its calculations, chromatographic column overloading will result in relatively lower peaks for the most abundant proteins and therefore always have a ratio of 1.

From nicely prepared samples with 0.1-0.2ug/ul peptide without interfering compounds you may get:

	Number of proteins quantified with a 1 hour gradient
Human blood serum	135
Human milk	185
Bacteria	800 - 1600
Bovine cell line	2500
Hela cell line	2500

C) Pricing

	Academic research	**Non-academic research**
MS rental and usage (SRF)	WUR: € 1040,- per day External: € 1352,- per day	€ 2080,- per day
nLC rental and usage	€ 75,- per day	€ 150,- per day
Sample preparation assistence	€ 325,- per half day *	€ 650,- per half day
Data analysis consisting of: database search, result filtering, prepare for and perform T-test(s) when applicable.	€ 400,- per measured day *	€ 1200,- per measured day
Report	Result will be handed in as a set of excel tables when data analysis was included	€ 1200,- for a full written report including set of excel tables

* Price reductions are possible for academic users when there is mutual agreemeent to consider the proteomics part as a scientific collaboration that may lead to a joined publication.

Some published articles with a proteomics contribution:

2019

Zeng, Z., E. Smid, S. Boeren, R. A. Notebaart and T. Abee (2019). "Bacterial microcompartment-dependent 1,2-propanediol utilization stimulates anaerobic growth of Listeria monocytogenes EGDe." Frontiers in Microbiology, section Microbial Physiology and Metabolism **10**(2660).

Elwakiel, M., S. Boeren, J. A. Hageman, I. M. Szeto, H. A. Schols and K. A. Hettinga (2019). "Variability of Serum Proteins in Chinese and Dutch Human Milk during Lactation." Nutrients **11**(3).

Florentino, A. P., I. A. C. Pereira, S. Boeren, M. van den Born, A. J. M. Stams and I. Sanchez-Andrea (2019). "Insight into the sulfur metabolism of Desulfurella amilsii by differential proteomics." Environmental Microbiology **21**(1): 209-225.

Göertz, G. P., J. v. Bree, A. Hiralal, B. M. Fernhout, C. Steffens, S. Boeren, T. M. Visser, C. B. Vogels, C. J. Koenraadt, M. M. v. Oers and G. P. Pijlman (2019 accepted). "Subgenomic flavivirus RNA binds the mosquito DEAD/H-box helicase ME31B and determines Zika virus transmission by Aedes aegypti." Proceedings of the National Academy of Sciences of the United States of America **116**(38): 19136-19144.

Mora, D., R. Filardi, S. Arioli, S. Boeren, S. Aalvink and W. M. de Vos (2019). "Development of omics-based protocols for the microbiological characterization of multi-strain formulations marketed as probiotics: the case of VSL#3." Microbial Biotechnology.

2018

Abdelkhaliq, A., M. van der Zande, A. Punt, R. Helsdingen, S. Boeren, J. J. M. Vervoort, I. M. C. M. Rietjens and H. Bouwmeester (2018). "Impact of nanoparticle surface functionalization on the protein corona and cellular adhesion, uptake and transport." Journal of Nanobiotechnology **16**.

Bao, Y., S. Boeren and P. Ertbjerg (2018). "Myofibrillar protein oxidation affects filament charges, aggregation and waterholding." Meat Science **135**: 102-108.

de Lamo, F. J., M. E. Constantin, D. H. Fresno, S. Boeren, M. Rep and F. L. W. Takken (2018). "Xylem Sap Proteomics Reveals Distinct Differences Between R Gene- and Endophyte-Mediated Resistance Against Fusarium Wilt Disease in Tomato." Frontiers in Microbiology **9**.

Florentino, A. P., I. A. C. Pereira, S. Boeren, M. v. d. Born, A. J. M. Stams and I. Sánchez-Andrea (2018). "Insights into sulfur reductive metabolism by differential proteomics of Desulfurella amilsii " Environmental Microbiology EMI-2018-0502.

Houbaert, A., C. Zhang, M. Tiwari, K. Wang, A. D. Serrano, D. V. Savatin, M. J. Urs, M. K. Zhiponova, G. E. Gudesblat, I. Vanhoutte, D. Eeckhout, S. Boeren, M. Karimi, C. Betti, T. Jacobs, C. Fenoll, M. Mena, S. de Vries, G. De Jaeger and E. Russinova (2018). "POLAR-guided signalling complex assembly and localization drive asymmetric cell division." Nature **563**(7732): 574-+.

Mishev, K., Q. Lu, B. Denoo, F. Peurois, W. Dejonghe, J. Hullaert, R. De Rycke, S. Boeren, M. Bretou, S. De Munck, I. Sharma, K. Goodman, K. Kalinowska, V. Storme, L. S. L. Nguyen, A. Drozdzecki, S. Martins, W. Nerinckx, D. Audenaert, G. Vert, A. Madder, M. S. Otegui, E. Isono, S. N. Savvides, W. Annaert, S. De Vries, J. Cherfils, J. Winne and E. Russinova (2018). "Nonselective Chemical Inhibition of Sec7 Domain-Containing ARF GTPase Exchange Factors." Plant Cell **30**(10): 2573-2593.

Pas, M. F. t., S. J. Koopmans, L. Kruijt, S. Boeren and M. A. Smits (2018). "Changes in Plasma Protein Expression Indicative of Early Diet-induced Metabolic Disease in Male Pigs (Sus scrofa)." Comparative Medicine.

Sedano-Nunez, V. T., S. Boeren, A. J. M. Stams and C. M. Plugger (2018). "Comparative proteome analysis of propionate degradation by Syntrophobacter fumaroxidans in pure culture and in coculture with methanogens." Environmental Microbiology **20**(5): 1842-1856.

Sousa, D. Z., M. Visser, A. H. van Gelder, S. Boeren, M. M. Pieterse, M. W. H. Pinkse, P. D. E. M. Verhaert, C. Vogt, S. Franke, S. Kummel and A. J. M. Stams (2018). "The deep-subsurface sulfate reducer Desulfotomaculum kuznetsovii employs two methanol-degrading pathways." Nature Communications **9**.

ten Klooster, J. P., A. Sotiriou, S. Boeren, S. Vaessen, J. Vervoort and R. Pieters

(2018). "Type 2 diabetes-related proteins derived from an in vitro model of inflamed fat tissue." Archives of Biochemistry and Biophysics **644**: 81-92.

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Benoit-Gelber, I., T. Gruntjes, A. Vinck, J. G. van Veluw, H. A. B. Wosten, S. Boeren, J. J. M. Vervoort and R. P. de Vries (2017). "Mixed colonies of Aspergillus niger and Aspergillus oryzae cooperatively degrading wheat bran." Fungal Genetics and Biology **102**: 31-37.

Brick, T., M. Ege, S. Boeren, A. Bock, E. von Mutius, J. Vervoort and K. Hettinga (2017). "Effect of Processing Intensity on Immunologically Active Bovine Milk Serum Proteins." Nutrients **9**(9).

Buntin, N., T. Hongpattarakere, J. Ritari, F. P. Douillard, L. Paulin, S. Boeren, S. A. Shetty and W. M. de Vos (2017). "An Inducible Operon Is Involved in Inulin Utilization in Lactobacillus plantarum Strains, as Revealed by Comparative Proteogenomics and Metabolic Profiling." Appl Environ Microbiol **83**(2).

Dingess, K. A., M. de Waard, S. Boeren, J. Vervoort, T. T. Lambers, J. B. van Goudoever and K. Hettinga (2017). "Human milk peptides differentiate between the preterm and term infant and across varying lactational stages." Food & Function **8**(10): 3769-3782.

Dongen, W. v., L. van Heerde, S. Boeren and S. C. de Vries (2017). "Identification of Brassinosteroid Signaling Complexes by Coimmunoprecipitation and Mass Spectrometry." Methods Mol Biol **1564**: 145-154.

Ottman, N., M. Davids, M. Suarez-Diez, S. Boeren, P. J. Schaap, V. A. P. M. dos Santos, H. Smidt, C. Belzer and W. M. de Vos (2017). "Genome-Scale Model and Omics Analysis of Metabolic Capacities of Akkermansia muciniphila Reveal a Preferential Mucin-Degrading Lifestyle." Applied and Environmental Microbiology **83**(18).

Ottman, N., J. Reunanen, M. Meijerink, T. E. Pietila, V. Kainulainen, J. Klievink, L. Huuskonen, S. Aalvink, M. Skurnik, S. Boeren, R. Satokari, A. Mercenier, A. Palva, H. Smidt, W. M. de Vos and C. Belzer (2017). "Pili-like proteins of Akkermansia muciniphila modulate host immune responses and gut barrier function." Plos One **12**(3).

Wendrich, J. R., S. Boeren, B. K. Möller, D. Weijers and B. D. Rybel (2017). "In Vivo Identification of Plant Protein Complexes Using IP-MS/MS." Methods in Molecular Biology **1497**(Plant Hormones: Methods and Protocols): 147-158.

Zwittink, R. D., D. van Zoeren-Grobben, R. Martin, R. A. van Lingen, L. J. G. Jebbink, S. Boeren, I. B. Renes, R. M. van Elburg, C. Belzer and J. Knol (2017). "Metaproteomics reveals functional differences in intestinal microbiota development of preterm infants." Molecular & Cellular Proteomics **16**(9): 1610-1620.

2016

Abd-Alla, A. M., H. M. Kariithi, F. Cousserans, N. J. Parker, I. A. Ince, E. D. Scully, S. Boeren, S. M. Geib, S. Mekonnen, J. M. Vlak, A. G. Parker, M. J. Vreysen and M. Bergoin (2016). "Comprehensive annotation of Glossina pallidipes salivary gland hypertrophy virus from Ethiopian tsetse flies: a proteogenomics approach." J Gen Virol **97**(4): 1010-1031.

Altena van , S. E., B. de Klerk, K. A. Hettinga, R. J. van Neerven, S. Boeren, H. F. Savelkoul and E. J. Tijhaar (2016). "A proteomics-based identification of putative biomarkers for disease in bovine milk." Vet Immunol Immunopathol **174**: 11-18.

Hollander den, P. W., P. D. G. Duarte, H. Bloksma, S. Boeren and J. W. M. van Lent (2016). "Proteomic analysis of the plasma membrane-movement tubule complex of cowpea mosaic virus." Archives of Virology **161**(5): 1309-1314.

Kar, S. K., A. J. M. Jansman, S. Boeren, L. Kruijt and M. A. Smits (2016). "Protein, peptide, amino acid composition, and potential functional properties of existing and novel dietary protein sources for monogastrics." Journal of Animal Science **94**: 30-39.

Kariithi, H. M., S. Boeren, E. K. Murungi, J. M. Vlak and A. M. Abd-Alla (2016).

"A proteomics approach reveals molecular manipulators of distinct cellular processes in the salivary glands of Glossina m. morsitans in response to Trypanosoma b. brucei infections." Parasit Vectors **9**(1): 424.

Kariithi, H. M., I. A. Ince, S. Boeren, E. K. Murungi, I. K. Meki, E. A. Otieno, S. R. G. Nyanjom, M. M. van Oers, J. M. Vlak and A. M. M. Abd-Alla (2016). "Comparative analysis of salivary gland proteomes of two Glossina species with differential hytrosavirus pathologies." Frontiers in Microbiology **7**.

Lu, J., N. Argov-Argaman, J. Anggrek, S. Boeren, T. van Hooijdonk, J. Vervoort and K. A. Hettinga (2016). "The protein and lipid composition of the membrane of milk fat globules depends on their size." Journal of Dairy Science **99**(6): 4726-4738.

Mehboob, F., M. J. Oosterkamp, J. J. Koehorst, S. Farrakh, T. Veuskens, C. M. Plugge, S. Boeren, W. M. de Vos, G. Schraa, A. J. M. Stams and P. J. Schaap (2016). "Genome and proteome analysis of Pseudomonas chloritidismutans AW-1(T) that grows on n-decane with chlorate or oxygen as electron acceptor." Environmental Microbiology **18**(10): 3247-3257.

Ottman, N., L. Huuskonen, J. Reunanen, S. Boeren, J. Klievink, H. Smidt, C. Belzer and W. M. de Vos (2016). "Characterization of Outer Membrane Proteome of Akkermansia muciniphila Reveals Sets of Novel Proteins Exposed to the Human Intestine." Frontiers in Microbiology **7**.

Tomita, S., I. C. Lee, S. van, II, S. Boeren, J. Vervoort, P. A. Bron and M. Kleerebezem (2016). "Characterisation of the transcriptional regulation of the tarIJKL-locus involved in ribitol-containing wall teichoic acid biosynthesis in Lactobacillus plantarum." Microbiology-Sgm **162**: 420-432.

Yi, L., M. A. J. S. v. Boekel, S. Boeren and C. M. M. Lakemond (2016). "Protein identification and in vitro digestion of fractions from Tenebrio molitor." European Food Research and Technology: 1-13.

Zhang, L., M. de Waard, H. Verheijen, S. Boeren, J. A. Hageman, T. van Hooijdonk, J. Vervoort, J. B. van Goudoever and K. Hettinga (2016). "Changes over lactation in breast milk serum proteins involved in the maturation of immune and digestive system of the infant." J Proteomics **147**: 40-47.

Zhang, L. N., S. Boeren, M. Smits, T. van Hooijdonk, J. Vervoort and K. Hettinga (2016)."Proteomic study on the stability of proteins in bovine, camel, and caprine milk sera after processing." Food Research International **82**: 104-111.

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Bui, T. P., J. Ritari, S. Boeren, P. de Waard, C. M. Plugge and W. M. de Vos (2015). "Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal." Nat Commun 6: 10062.

Hettinga, K. A., F. M. Reina, S. Boeren, L. N. Zhang, G. H. Koppelman, D. S. Postma, J. J. M. Vervoort and A. H. Wijga (2015). "Difference in the Breast Milk Proteome between Allergic and Non-Allergic Mothers." Plos One 10(3).

Zhang, L., S. Boeren, J. A. Hageman, T. van Hooijdonk, J. Vervoort and K. Hettinga (2015). "Bovine milk proteome in the first 9 days: protein interactions in maturation of the immune and digestive system of the newborn." PLoS One 10(2): e0116710.

De Rybel, B., Moller, B., Yoshida, S., Grabowicz, I., de Reuille, P.B., Boeren, S., Smith, R.S., Borst, J.W. and Weijers, D. (2013) A bHLH Complex Controls Embryonic Vascular Tissue Establishment and Indeterminate Growth in Arabidopsis. Developmental Cell 24(4), 426-437.

Sotoca, A.M., Roelofs-Hendriks, J., Boeren, S., van der Kraan, P.M., Vervoort, J., van Zoelen, E.J.J. and Piek, E. (2013) Comparative proteome approach demonstrates that platelet-derived growth factor C and D efficiently induce proliferation while maintaining multipotency of hMSCs. Experimental Cell Research 319(17), 2649-2662.

Smaczniak, C., Li, N., Boeren, S., America, T., van Dongen, W., Goerdayal, S.S., de Vries, S., Angenent, G.C. and Kaufmann, K. (2012) Proteomics-based identification of low-abundance signaling and regulatory protein complexes in native plant tissues. Nature Protocols 7(12), 2144-2158.

Hettinga, K., van Valenberg, H., de Vries, S., Boeren, S., van Hooijdonk, T., van Arendonk, J. and Vervoort, J. (2011) The Host Defense Proteome of Human and Bovine Milk. Plos One 6(4).